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Special Report:

Workshop on Autoimmune (Idiopathic) Thrombocytopenic Purpura: Pathogenesis and New Approaches to Therapy

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Autoimmune (idiopathic) thrombocytopenic purpura (ITP) was first recognized as a disease entity more than 100 years ago. A fatal outcome was not uncommon until the benefits of splenectomy were recognized early in this century. When corticosteroids became available in the late 1940s, it became possible to manage most patients successfully by administration of these agents with or without surgery. However, a subset of children and adults unresponsive to this regimen continue to pose therapeutic challenges. It is now recognized that autoimmune thrombocytopenia is a common complication of HIV-1 infection, and the molecular mechanisms underlying this relatively common autoimmune disorder remain poorly understood. In August 1997, the National Heart, Lung, and Blood Institute sponsored a workshop to review current understanding of the pathogenesis of ITP and to discuss research initiatives to address unresolved questions concerning this disorder (or more accurately, this group of disorders).

A TRIBUTE

The meeting began with a presentation by *Richard Aster (Medical College of Wisconsin),* crediting contributions made by the late William J. Harrington and N. Raphael Shulman, who, through clinical investigations carried out in the 1950s and 1960s, established ITP as an antibody-mediated autoimmune disorder in which sensitized platelets are destroyed in the reticulo-endothelial system, notably, in the spleen.

NORMAL IMMUNE RESPONSE AND AUTOIMMUNITY

Ethan Shevach (National Institute of Allergy and Infectious Diseases) reviewed evidence that self-reactive T cells are normally found in peripheral lymphoid tissues where they persist in a state of ''indifference/ignorance.'' Latent autoreactivity can become manifest when autoantigen becomes available in an immunogenic form or the immune system sees infectious organisms that carry epitopes cross-reactive with epitopes of the host. The possible relationship of cytokines produced in response to infectious agents to the promotion of autoimmunity was reviewed. The relatively low incidence of autoimmune diseases in the general population indicates the existence of mechanisms that ordinarily hold autoreactive T cells in check. *David Parker (University of Oregon)* briefly summarized the current understanding of T- and B-cell interactions in immunity and tolerance with emphasis on the importance of CD40 as a receptor for cell contact-dependent T-cell help. This interaction enables T cells to induce expression in B cells of co-stimulatory signals needed to respond positively to antigen. *Stephen D. Miller (Northwestern University Medical School)* discussed the rule of ''epitope spreading'' in the pathogenesis of autoimmune and virus-induced disorders and reviewed animal models of demyelinating disease in which this phenomenon appears to be central to disease progression. It is now recognized that T-cell responses diversify over the course of chronic organ-specific autoimmune diseases in response to the release of normally sequestered antigens. In experimental autoimmune encephalomyelitis, a dominant peptide of myelin basic protein initiates the autoimmune response, but relapses are mediated by CD4-positive T cells specific for other, endogenous myelin epitopes made available to the immune system during acute tissue destruction. In multiple scle-

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rosis, CNS damage may be perpetuated by CD4 positive T cells responding initially to a persistent virus infection of the CNS. ''Epitope spreading'' appears to provide an alternative to molecular mimicry as an explanation for the development of infection-induced, organ-specific autoimmunity. *John Semple (Departments of Pharmacology and Medicine, University of Toronto)* described recent studies in which CD4- and CD8-positive cell lines were derived from spleen cells of patients with chronic ITP as starting material. The T-cell lines are specifically stimulated by autologous platelets but only in the presence of adherent CD14-positive splenic feeder cells. An understanding of the activation requirements of these T cells may lead to identification of autoantigens important in ITP and point the way toward specific types of immunotherapy. *Diane Nugent (Children's Hospital of Orange County)* reviewed the role of TH1 and TH2 helper cells in the immune response and discussed studies indicating that peripheral blood mononuclear cells of children with AITP are profoundly deficient in IL-4 production at presentation. TH_1 or TH_2 dominance may influence the cytokine milieu, which, in turn, controls class switching of immunoglobulin molecules and continued expansion of B cells producing platelet-specific autoantibodies.

THROMBOKINETICS

David Kuter (Massachusetts General Hospital) reviewed megakaryocytopoiesis and the regulation of platelet production. Thrombopoietin (TPO) is now known to be the key regulator of thrombopoiesis. In the absence of TPO or its receptor, Mpl, megakaryocytes are reduced and platelets are produced at a rate only about 15% of normal. TPO produced constitutively by the liver normally binds to Mpl expressed on circulating platelets and is cleared from the circulation. In patients with ITP, TPO levels are normal or slightly elevated, in contrast to the high levels seen in patients with suppressed platelet production. This may be due to inadequate TPO production in ITP or to clearance and metabolism of TPO by an increased mass of megakaryocytes and the increased numbers of platelets they produce. Accordingly, it is uncertain whether TPO administration will be helpful in AITP. *Terry Gernsheimer (Puget Sound Blood Center)* discussed platelet kinetics in ITP. Increased numbers of megakaryocytes normally found in the marrow, have led to the view that thrombocytopenia is primarily a consequence of peripheral destruction of platelets released from the marrow at a normal or above normal rate. However, the results of platelet survival studies using labeled, autologous platelets are consistent with the possibility that platelet production is subnormal in some patients. Following treatment with corticosteroids, the rate of platelet production appears to increase. Splenectomy appears to exert its beneficial effects mainly by prolonging platelet survival. There followed a discussion of whether kinetics of radiolabeled autologous platelets provide a valid index of platelet production, and it was agreed that more information about production and destruction of platelets in ITP is needed.

PATHOGENESIS OF AITP AND SIMILAR IMMUNE DISORDERS

Robert McMillan (Scripps Research Institute) reviewed the characteristics of platelet-specific autoantibodies associated with AITP and the targets they recognize. Most autoantigens are carried on the platelet membrane glycoprotein (GP) complexes IIb/IIIa or Ib/IX, the most abundant of platelet membrane glycoproteins. Recently, the GPIa/IIa complex has also been implicated. Although most autoantibodies recognize extracellular regions of these glycoproteins, some appear to be specific for the cytoplasmic (C-terminal) portion of GPIIIa. Possibly, these autoantibodies arise secondarily when sensitized platelets are sequestered in large numbers in the spleen. Most autoantibodies specific for GPIIb/IIIa appear to recognize epitopes found only on the cation-dependent, intact GPIIb/IIIa complex. In a few instances, however, autoantibodies have been shown to recognize linear peptide sequences. *Thomas Kunicki (Scripps Research Institute)* further reviewed the status of GPIIb/IIIa (integrin $\alpha IIb\beta 3$) as a common target for platelet-reactive auto-, allo-, and drug-induced antibodies. Several monoclonal human antibodies reactive with GPIIb/IIIa have been shown to have idiotypes found commonly among human autoantibodies specific for this GP complex, but not found in pools of naturally occurring antibodies or antibodies generated in other immune responses to GPIIb/IIIa. He also discussed integrin $\alpha_2\beta_1$ (GPIa/IIa) as a target for autoantibodies in some patients with ITP, and reviewed his recent observations indicating that expression of $\alpha_2\beta_1$ varies markedly in normal subjects on a genetic basis. *Thomas Kickler (Johns Hopkins University)* reviewed immunologic methods used for the diagnosis of AITP. Early studies in which methods suitable for the detection of red cell antibodies were used to detect platelet-reactive antibodies in serum, failed to shed much light on platelet-specific autoantibodies. Alternative methods employed in recent years have focused on detection of platelet-associated autoantibodies. Although current methods are sensitive, they are lacking in specificity, because platelet-associated immunoglobulin levels are elevated in various nonimmune thrombocytopenic disorders. Attempts to improve the specificity of assays have been frustrated by the fact that the alpha granules of platelets contain significant amounts of normal plasma IgG and by the tendency of platelets to absorb IgG, nonspecifically, especially in the form of immune complexes. Recent emphasis has been placed on assays in which platelet glycoproteins known to be targets for au-

toantibodies are detergent-solubilized and captured by immobilized monoclonal antibodies. Autoantibody associated with these glycoproteins is then detected by ELISA. An alternative approach is to prepare an eluate from the patient's own platelets and test it for specificity against normal platelet glycoproteins. Although these approaches appear promising, a ''gold standard'' assay is not yet available. *Richard Aster (Blood Center of Southeastern Wisconsin and Medical College of Wisconsin)* discussed the possible role of drugs and other exogenous substances in the pathogenesis of autoimmune thrombocytopenia. Many drugs are capable of inducing a remarkable class of antibodies that binds to platelet glycoproteins only in the presence of the provocative drug in solution. A subset of such patients simultaneously produces true autoantibodies that bind to platelet glycoproteins in the absence of drug. In some instances, these autoantibodies persist and continue to perpetuate thrombocytopenia even in the absence of further drug exposure. It was speculated that some cases of ITP may be triggered by exposure to exogenous substances such as bacteria and viruses. *Simon Karpatkin (New York University Medical Center)* discussed ITP as a common complication of HIV-1 infection. HIV-1/ITP behaves clinically like other forms of ITP. However, patients with HIV-1 infection have higher levels of platelet-associated immunoglobulins and demonstrable, circulating immune complexes. An anti-GPIIIa antibody isolated from these serum immune complexes appears to be specific for GPIIIa residues 49–66. Antibodies specific for this peptide bind to platelets and then react with rheumatoid factor and/anti-idiotypic antibody to fix complement and opsonize platelets for destruction.

CLINICAL ASPECTS OF ITP

James George (University of Oklahoma) discussed practice guidelines developed recently by a committee of the American Society of Hematology. He stressed the lack of definitive diagnostic tests to distinguish ITP from other forms of thrombocytopenia in children and adults. In the last analysis, the diagnosis of ITP is currently based on history, physical examination, routine blood counts, examination of a peripheral blood smear, and, where necessary, bone marrow examination. In discussion, the need for more specific diagnostic tools was emphasized. *Jeanne Lusher (Children's Hospital of Michigan)* discussed ITP in childhood. She noted that childhood ITP is generally a benign, self-limited disorder with complete spontaneous recovery occurring within days to months. Thrombocytopenia often occurs in the wake of a viral infection and, although bleeding symptoms may initially be severe, supportive measures such as protection from head injury during the acute phase will usually suffice. Whether administration of corticosteroids and/or intravenous gamma globulin is beneficial is controversial. An individualized approach is most appropriate. A small percentage of children with ITP fail to achieve remission and enter a chronic phase in which they respond to the same treatment regimes that are effective in adults. However, splenectomy should be avoided if at all possible in children less than 4 years of age because the operation is known to predispose them to catastrophic bacterial infection. *James Bussel (New York Hospital-Cornell Medical Center)* then reviewed treatment alternatives in childhood ITP and noted the rapid rise in platelet levels that usually follows administration of high dose corticosteroids or IV IgG. He agreed with Dr. Lusher that conservative management is appropriate, but observed that many clinicians believe active treatment is indicated to reduce the risk of intracranial hemorrhage. Primary treatment options include corticosteroids and/or intravenous gamma globulin. IV anti-Rho D immunoglobulin, especially at a dose of 75 μg/kg, also appears effective and is less costly than gamma globulin. No markers to predict the risk of hemorrhage or chronicity in childhood ITP have yet been identified. *Douglas Cines (University of Pennsylvania)* noted that very few properly controlled clinical trials of the treatment of ITP in adults have yet been carried out. Thus, most current practices have evolved from empiric observation alone. Even the natural history of the disease is not adequately defined, and there is little consensus on the fraction of patients that achieve complete remission on supportive treatment with corticosteroids alone. Most patients treated with corticosteroids experience a rise in the platelet count to levels at which the risk of intracranial hemorrhage is small. Many eventually undergo splenectomy because of intolerance to the dose of steroids required to maintain platelets at satisfactory levels. A high percentage of younger patients experience complete remission after splenectomy. The incidence of complete remission is lower in older patients, but even poor responders can usually be maintained on lower doses of corticosteroids after surgery. Second-line therapies for ITP include intravenous IgG, anti-Rho immunoglobulin, vinca alkaloids, danazol, and dapsone, which are thought to inhibit phagocytosis of platelets by macrophages, thus impairing their clearance, and cyclophosphamide, azathioprine, and cyclosporin, for the purpose of immunosuppression. Whether perfusion of patient plasma on a staphylococcal protein A column is effective is controversial. Serologic studies, measurements of platelet kinetics, and determination of the sites of platelet destruction have not been shown to be helpful in planning treatment or assessing prognosis. In discussion, it was agreed that there is a need for controlled, prospective studies of the management of both newly diagnosed patients and patients refractory to conventional therapy.

EXPERIMENTAL APPROACHES TO TREATMENT

As in any autoimmune disease, collaboration of T lymphocytes with other cells in patients with ITP depends on interactions mediated through both the antigen-specific T cell receptor (TCR) and antigen-nonspecific accessory molecules. One important accessory molecule is the T-cell CD40 ligand (CD40L, CD144). CD40L is transiently expressed on helper T ($CD4^+$) lymphocytes following early activation, usually mediated through the TCR and plays an important early role in the interaction of activated T cells with cells expressing CD40, including B lymphocytes, macrophages, and dendritic cells. In general, this interaction leads to activation of the CD40 bearing cell. *Michael Kauffman (Biogen Corporation)* reported that Biogen has developed a ''humanized'' monoclonal antibody specific for CD40L that interferes with this interaction. Studies of autoimmune disease models in animals have demonstrated safety and efficacy of this approach. A Phase I study in chronic ITP has been initiated and Phase II studies are planned. *Sharon Cochran (Cypress Bioscience, Inc.)* reviewed the use of Protein A immuno-absorption in the treatment of adult ITP. Treatment consists of passing various amounts of patient plasma over the column ex vivo, followed by reinfusion into the donor. This process is known to remove various amounts of IgG and, especially, immune complexes from the perfusate, but the mechanism by which passage of only a fraction of a patient's plasma over such a column might lead to clinical improvement is uncertain. Nonetheless, patients with chronic ITP resistant to other treatments have experienced increases in platelet counts following such treatment, and some appear to have achieved complete remission.

CLINICAL TRIALS IN ITP

Gary Raskob (University of Oklahoma Health Sciences Center) observed that the management of patients with ITP is fraught with uncertainty for the practicing clinician because of a lack of rigorous clinical trial data on which to base decisions for patient care and noted that current uncertainties can only be solved by performing adequately designed and executed clinical trials. The wealth of reports in the literature describing uncontrolled series of patients with ITP indicates that enough patients should be available to allow coordinated, multi-center clinical trials to be conducted. *James George (University of Oklahoma)* reported that a network of hematologists has been established by his group at the University of Oklahoma Medical School to facilitate clinical trials in ITP. He expressed the opinion that methods utilized by national cooperative groups concerned with oncology can be applied to gain valuable information about the natural history and long-term outcomes of patients with non-malignant conditions such as ITP. *Robert McMillan (Scripps Research Institute)* briefly described an ''ITP study group,'' initially organized in 1996 with the aim of designing and implementing clinical trials of treatment in chronic ITP. Sixteen centers, each headed by a physician with a personal interest in ITP, have been enrolled. Two protocols have already been developed. The first involves evaluation of the effectiveness of megakaryocyte growth and development factor (MGDF, TPO) in the treatment of patients with chronic ITP. The second is an evaluation of high-dose dexamethazone in the treatment of patients pre-splenectomy. The latter therapy has proved remarkably effective in some studies, but ineffective in others, and it is hoped that a prospective, controlled study will help to define its place in the treatment of ITP. *James Bussel (New York Hospital–Cornell University Medical Center)* reviewed clinical trials performed in children with acute ITP. In many of these trials, early use or non-use of prednisone has been compared. Other studies have compared IV gamma globulin to prednisone to no treatment, and various doses of IV gamma globulin to anti-Rho immunoglobulin. The high rate of spontaneous improvement in children with acute ITP has complicated the evaluation of these treatments. In general, anti-Rho, high-dose corticosteroids, and IV gamma globulin appear to shorten the period of severe thrombocytopenia. Whether this translates into a reduced risk of intracranial hemorrhage is uncertain, however, because of the rarity of this complication.

SUMMARY

It was the consensus of Workshop participants that ITP, the most common autoimmune disorder affecting a blood element, continues to present challenging opportunities for basic and clinical research. These include: studies at the molecular level to characterize the autoimmune response and the possible contribution of exogenous agents such as viruses (especially HIV), drugs, and environmental exposures; development of animal models of autoimmune thrombocytopenia and use of these to study pathogenesis; evaluation of thrombokinetics and megakaryocytopoiesis to characterize the relative importance of platelet destruction and megakaryocyte suppression to the development of thrombocytopenia and to evaluate the therapeutic effectiveness of thrombopoietic hormone administration; development of more sensitive and specific methods for diagnosis and better characterization of the responsible autoantibodies and the target epitopes they recognize; application of in vitro assays to distinguish between acute and chronic forms of ITP, HIV-associated and non-HIV-associated ITP, and the response of these classes of patients to treatment; studies of the mechanism of action of intravenous gamma globulin and other therapeutic modalities and the development of safe and effective methods to interfere with autoantibody production.